Appendix 5: Summary of available bee toxicity data for thiamethoxam

This appendix includes a summary of the bee toxicity data available for thiamethoxam from registrant submitted studies and the scientific literature. The appendix describes Tier I (individual level laboratory toxicity studies), Tier II (semi field tunnel or feeding studies) and Tier III (full field) studies, focusing on endpoints that are relevant to survival, growth or reproduction of individuals or colonies.

Tier I

Adult Acute Contact Toxicity

***Apis* – Registrant-Submitted Studies**

Several studies are available to characterize the acute contact toxicity of thiamethoxam to honey bee adults (**Table 1**). Studies include TGAI as well as several formulated products. The LD50 values for contact exposure range 0.02-0.39 µg c.e./bee. Comparison of LD50 values for TGAI and formulated products indicate that there is no substantial difference in toxicity for four formulated products, with the TGAI LD50 of 0.021 µg c.e./bee, being within an order of magnitude of four different formulated products. The TGAI LD50 is an order of magnitude less sensitive than the LD50 for Actara® and an order of magnitude more sensitive than the LD50 for Actara® 75 WG.

Table 1. Thiamethoxam Tier I acute contact toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration) reported in terms of thiamethoxam active ingredient and clothianidin equivalents (c.e.)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Thiamethoxam® WG (25)	0.019 (0.014-0.024)	0.016	49950111	Acceptable
Thiamethoxam® 240SC (21.6)	0.0198 (0.0163-0.0237)	0.0169	49950105	Acceptable
TGAI (98.6)	0.024 (0.021-0.027)	0.021	44714927	Acceptable
Cruiser® 600 FS (NA)	0.066 (0.012-1093)	0.056	49950114	Supplemental (qualitative)
Actara® 75 WG (74.8)	0.46 (0.34-0.68)	0.39	49950106	Acceptable
Thiamethoxam® WG (25)	23.5 (22.2-28.7) 48-hr LC_{50}	20.1	49950119	Supplemental (qualitative)
Thiamethoxam Cruiser 350 FS	Not Calculated**	Not Calculated**	49950122	Supplemental (qualitative)
Thiamethoxam Formulation*	0.5 formulation (0.37-0.69)	0.428	49950116	Unacceptable

*This formulation contained 81.9 g a.i./L. The results were reported in terms of mg wm/mL. "WM" meant "whole material" which were presumed to be formulation. It was not clear if the liquid formulation was weighed or if the weight of thiamethoxam was calculated when making the dosing solutions. The authors did note they were not adjusted for purity.

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**There were only 2 doses tested

***Apis* – Open Literature Studies**

The open literature studies considered include two acute, contact-based studies with adult honey bees (**Table 2**), both involving TGAI. The LD50 value generated by Iwasa *et al.* (2004), *i.e.*, 0.0256 µg c.e./bee, is similar to the registrant-submitted study with TGAI (LD50 0.021 µg c.e./bee; MRID 44714927). The LD50 value reported by Thompson *et al.* is 5-fold greater than the other two TGAI values.

Table 2. Thiamethoxam Tier I acute contact toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
TGAI (>99)	0.0299 (NA)	0.0256	Iwasa *et al.* 2004	Qualitative
TGAI (99.7)	0.124 (0.0768-0.328)	0.106	Thompson *et al.* 2014	Qualitative

NA = not applicable

Non-*Apis* – Registrant-Submitted studies

One registrant-submitted study is available for adult bumble bees (B. *terrestris* (L.)) exposed to thiamethoxam via contact (**Table 3**). The contact LD50 value is 0.094 µg c.e./bee (MRID 49950109). This LD50 is an order of magnitude higher (*i.e.*, less sensitive) than the honey bee value for the same formulated product (*i.e.*, LD50 = 0.00475 µg c.e./bee; MRID 49950125).

Table 3. Summary of registrant submitted adult acute contact toxicity studies for non-Apis bees (*Bombus terrestris terrestris*) exposed to thiamethoxam

Test material (% a.i.)	Study Duration (Type)	LD_{50} Thiamethoxam (95% CI; units: µg a.i./bee)	Clothianidin equivalents	Comments	Classification (Reference, MRID)
Actara 25 WG (25.2)	72-hr	0.11 (0.10-0.13)	0.094	none	~~Supplemental--Quantitative~~Acceptable (49950109)

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Non-*Apis* – Open Literature Studies

Sechser *et al.* 2002 exposed bumble bees (B. *terrestris* L.) to thiamethoxam (Actara™ WG 25) via contact with glass plates that were sprayed at levels representative of an application rate of 8.6 g c.e./ha. All exposed bees died within 7 d. The doses received by bees were not quantified (**Table 4**).

Valdovinos-Núñez *et al.* (2009) exposed stingless bees (*Nannotrigona perilampoides*) to thiamethoxam (TGAI) via contact exposure at levels of 0.009, 0.09, 0.4 and 0.9 µg c.e./bee. After 24 hours, the LD50 was 0.003 µg (95% CI: 0.002-0.005) c.e./bee; however, there is considerable uncertainty associated with this endpoint as it is below the lowest level tested.

Table [STYLEREF 1 \s]. Summary of open literature adult acute contact toxicity studies for non-Apis bees exposed to thiamethoxam.

Test material (% a.i.)	Study Duration (Type)	LD_{50} Thiamethoxam (95% CI; units: µg a.i./bee)	Clothianidin equivalents s	Test Organism	Classification (Reference, MRID)
Actara™ WG 25 (25)	7 d	10 g c.e./ha	8.6 g c.e./ha	Bumble bee *Bombus terrestris*	Qualitative (Sechser *et al.* 2002)
TGAI	24 h	0.004 (0.003-0.006)	0.003	Stingless bees (*Nannotrigona perilampoides*)	Qualitative (Valdovinos-Núñez *et al.* (2009)

Adult Acute Oral Toxicity

***Apis* – Registrant-Submitted Studies**

Several studies are available to characterize the acute oral toxicity of thiamethoxam to honey bee adults (**Table 5**). Studies include TGAI as well as several formulated products. Comparison of LD50 values for TGAI and formulated products indicate that there is no substantial difference in toxicity (all within the same order of magnitude). The LD50 values for oral exposure range from 0.0031 to 0.006794 µg a.i./bee (or 0.0026-0.00578 µg c.e./bee as clothianidin equivalents). These data indicate that thiamethoxam is more toxic to bees exposed through diet compared to through direct contact exposure.

Table 5. Thiamethoxam Tier I acute oral toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration) expressed in terms of active ingredient (a.i.) and clothianidin equivalents (c.e.)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Thiamethoxam® 240SC (21.6)	0.00309 (0.00256-0.00366)	0.00265	49950105	Acceptable
TGAI	0.0044 (NA)	0.0038	49005702	Acceptable
TGAI (98.6)	0.005 (0.004-0.006)	0.004	44714927	Acceptable
~~Actara® (25.2)~~	~~0.00631 (NA)~~	~~0.00540~~	~~49950125~~	~~Supplemental (qualitative)~~
Formulated product (20.6% thiamethoxam, 20.6% cyantraniliprole)	0.0064* [0.031 µg test material/bee]	0.0055	48432530	Acceptable
Thiamethoxam® SG (72.8)	0.00668 (0.00571-0.00773)	0.00572	49950115	~~Supplemental (qualitative)~~Acceptable
~~Dust (from Cruiser® 250 FS, 7.24)~~	~~0.00936 (NA)~~	~~0.00801~~	~~49950125~~	~~Supplemental (qualitative)~~
Thiamethoxam Cruiser 350 FS	Not Calculated**	Not Calculated**	49950122	Supplemental (qualitative)
Thiamethoxam Formulation*	0.085 (0.065-0.11)	0.073	49950116	Unacceptable

NA = Not Applicable

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*This formulation contained 81.9 g a.i./L. The results were reported in terms of mg wm/mL. "WM" meant "whole material" which were presumed to be formulation. It was not clear if the liquid formulation was weighed or if the weight of thiamethoxam was calculated when making the dosing solutions. The authors did not they were not adjusted for purity.
**There were only 2 doses tested.

***Apis* - Open Literature Studies**

Three qualitative studies are considered from the literature (**Table 6**). The LD_{50} values are within the range of the registrant-submitted LD_{50} values reported above.

Table 6. Thiamethoxam Tier I acute oral toxicity data for adult honey bees (*Apis mellifera*) (48-h study duration) expressed in terms of active ingredient (a.i.) and clothianidin equivalents (c.e.)

Test material (% a.i.)	LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Actara® 25 WG	0.0026-0.0044 (NA)	0.0022-0.0038	Laurino *et al.* 2010	Qualitative
TGAI (92.6)	0.00428 (NA)	0.00366	Oliveria *et al.* 2013	Qualitative
TGAI (99.7)	0.0112 (0.00915-0.0135)	0.00959	Thompson *et al.* 2014	Qualitative

NA = Not Applicable

Non-*Apis* – Registrant Submitted Studies

One registrant submitted study is available for adult bumble bees (*Bombus terrestris* (L.)) exposed to thiamethoxam (**Table 7**). This study determined an acute oral LD_{50} value of 0.017 µg c.e./bee (MRIDs 49950107).

Table 7. Summary of registrant submitted adult acute oral toxicity studies for non-Apis bees (Bombus terrestris terrestris) exposed to thiamethoxam expressed in terms of active ingredient (a.i.) and clothianidin equivalents (c.e.)

Test material (% a.i.)	72-hr LD_{50} Value (95% CI; units: µg a.i./bee)		MRID/source	Classification
	Thiamethoxam	Clothianidin equivalents		
Actara 25 WG (25.2)	0.02	0.017	49550107	Acceptable~~None~~

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Non-Apis – Open Literature Studies

Sechser et al. 2002 exposed bumble bees (B. terrestris (L.)) to thiamethoxam (Actara® WG 25) via dietary exposure at levels representative of an application rate of 8.6 g c.e. /ha. All exposed bees died within 7-d (Table 8).

Table 8. Summary of open literature adult acute oral toxicity studies for non-Apis bees exposed to thiamethoxam

Test material (% a.i.)	Study Duration (Type)	Thiamethoxam (95% CI)	Clothianidin equivalents	Test Species	Classification (Reference, MRID)

		(expressed in terms of µg c.e./bee)			
Actara WG 25 (25)	7 d	10 g c.e./ha	8.6 g c.e./ha	*Bombus terrestris*	Qualitative (Sechser et al. 2002)

Adult chronic oral toxicity

Apis

Seven chronic toxicity studies are available for honey bees exposed to thiamethoxam (**Table 9**). One study is available for deriving risk quotients while the remaining six have limitations such that they are useful for characterizing potential effects of thiamethoxam on bees. All studies were conducted in a laboratory with either *A. mellifera* or the Indian honey bee *A. cerana indica*. The majority of the studies involved exposures via diet (oral exposure to spiked sucrose solution). Several of these studies describe effects related to sublethal endpoints with unknown links to apical endpoints (*i.e.*, survival growth or reproduction of individuals or hives). Of all the studies, effects to apical endpoints were observed in three studies: at 212 mg a.i/kg solution (181 mg c.e./kg solution), 70.3% mortality was observed (MRID 50084901), 428 µg a.i./L (366 µg a.i./L clothianidin- equivalents), bee lifespan was reduced by 41% (Oliveria *et al*. 2013) and at 500 µg a.i./L (428 µg a.i./L clothianidin-equivalents), 25% mortality was observed (Chandramani *et al*. 2008).

Chronic oral toxicity data for adult honey bees (A. *mellifera* L.) are available from three registrant-submitted studies (**Table 9**). In these studies, bees were dosed for 10 days through sucrose solution. In MRID 50084901 significant effects (relative to the control), on mortality was observed at 4.87 ng a.i/bee/day (LOAEC), while food consumption was affected at 1.84 ng a.i./bee/day (4.2 and 1.6 ng c.e./bee/day respectively). No effects were observed in the remaining studies, with the highest tested doses being 0.002 and 0.008 µg c.e./bee (MRIDs 49950110 and 49346603), which correspond to dietary concentrations of 8.6 and 27 µg c.e./L.

~~In a 5-d oral toxicity study with Indian honey bees (*A. cerana indica* F.), bees fed 500 µg/L thiamethoxam (430 µg/L clothianidin equivalent) experienced 25% mortality (Chandramani *et al*. 2008). One major uncertainty associated with the results of this study is that the test material is unclear in the article; therefore, it is unknown whether the endpoint was from a formulated product or TGAI and whether or not the concentration was adjusted to active ingredient.~~

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In a chronic study with Africanized honey bees (A. *mellifera*, Oliveira *et al* 2013), honey bees (newly emerged worker) exposed for 18 days to 366 µg c.e./L diet thiamethoxam through sucrose had a reduced lifespan (in days). In this exposure, 50% of bees lived 8d in the control; whereas, 50% of bees exposed to thiamethoxam only lived 5.2 days, resulting in a 41% decrease in the lifespan of adult worker bees. Bees exposed for 8 to 36.6 µg c.e./L diet had morphological changes (histological changes in neural mushroom bodies and optical lobes) of the brain and chemical changes (cytotoxicity) to the midgut. Similar to Oliveria *et al*. 2013, the study by Catae *et al*. 2014 exposed Africanized honey bees for 8 d to 36.6 µg c.e./L. Damage (cytotoxicity) to the midgut and Malpighian tubules were reported.

Aliouane *et al*. 2009 exposed adult bees to thiamethoxam via oral or contact exposure at levels of 0.00009 and 0.0009 µg c.e./bee. At the lower level, bees exposed via contact showed a decrease in olfactory memory (via testing proboscis extension reflex), relative to the control (p=0.02). At the higher level, bees exposed via contact had impaired learning (two trials p=0.025,0.033). Also at the higher level, bees

exposed via diet had a decrease in proboscis extension reflex (PER) when stimulated with sucrose. This study focused on sublethal effects; however, without information of how these effects related to survival, growth or reproduction of individuals or the colony, the relevance of these effects to the individual bee or colony is unknown.

Table 9. Laboratory chronic toxicity data for adult honey bees (Apis sp.)

Test dose (µg a.i./bee)		Test concentration (ng /g)[a]		Exposure route	Test material (% a.i.)	Duration (d)	Observed effects	Source	Classification
Thia-methoxam	Clothianidin-equivalent	Thia-methoxam	Clothianidin-equivalent						
0.0025/0.0049	0.0021/0.0042	120/212	103/181	Oral*	TGAI (99.5)	10	Mortality	50084901	Acceptable
0.0001	0.000086	NA	NA	Contact	TGAI (97)	11	Decrease in olfactory memory	Aliouane *et al* 2009 (MRID 47800507)	Qualitative
0.001	0.00086	NA	NA	Contact	TGAI (97)	11	Learning impairment	Aliouane *et al* 2009 (MRID 47800507)	Qualitative
0.001	0.00086	NA	NA	Oral*	TGAI (97)	11	Decrease in proboscis extension response to sucrose stimulation	Aliouane *et al* 2009 (MRID 47800507)	Qualitative
0.002	0.0017	10 µg/L	8.6 µg/L	Oral*	TGAI (99)	10	No effects to mortality or food consumption observed. No LOAEC was established.	MRID 49950110	Supplemental (qualitative)
0.00898	0.00768	27	23	Oral*	TGAI (99)	10	No effects to mortality or food consumption observed. No LOAEC was established.	MRID 49346603	Supplemental (qualitative)
NA	NA	42.8 µg/L	36.6 µg/L	Oral*	TGAI (92.5)	8	Cytotoxicity observed in midgut and Malpighian tubules	Catae *et al.* 2014	Qualitative
NA	NA	42.8 µg/L	36.6 µg/L	Oral*	TGAI (92.5)	8	Morphological changes to brain and chemical changes to midgut	Oliveira *et al.* 2013	Qualitative
NA	NA	428 µg/L	366 µg/L	Oral*	TGAI (92.5)	18	Reduced lifespan (41 % reduction)	Oliveira *et al.* 2013	Qualitative
~~NA~~	~~NA~~	~~500 µg/L~~	~~428 µg/L~~	~~Oral*~~	~~unknown~~	~~5~~	~~25% mortality~~	~~Chandramani *et al.* 2008 (MRID 49750602)~~	~~Qualitative~~

*Bees were fed sucrose solution. NA = not available

[a] Unless specified data are in ng/g.

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Larval Toxicity

Apis

Several studies are available to characterize the toxicity of thiamethoxam (TGAI) to honey bee larvae (**Table 11**). MRID 50096607 is an acceptable larval chronic toxicity study for which the acute endpoint was extrapolated. Effects were seen on adult emergence at day 22 and pupal mortality at day 15, with no effects (>50%) to larval mortality seen at day 8. The remaining studies are considered scientifically valid, but have notable limitations that prevent quantitative use of these data (*i.e.*, to derive RQs). Two studies evaluated impacts on larval survival following repeated dietary doses, generating a 48-hour LC_{50} value of 11.7 and a 7-day LC_{50} 23 µg a.i./g-diet and a 7-day LD_{50} of 0.78 µg a.i./larva/day (Tavares *et al.* 2015 and MRID 49950118). Data from a third acute toxicity study (MRID 49346602) failed to generate a definitive LC_{50} with only 29% mortality observed at the highest test level of 113 µg a.i./g diet, which was an order of magnitude above the LC_{50} values estimated for the other two studies. In a chronic repeat-dose study (22-D), significant mortality (12 and 16%) was observed at 0.025 and 0.050 µg a.i./g-diet (respectively), resulting in a 22-day NOAEC of 0.0125 µg a.i./g- diet (MRID 49513601). It is notable that the dose-response observed in this study was very shallow, as mortality only increased 4% relative to controls, despite a two-fold increase in exposure.

Table 11. Tier 1 Acute and Chronic toxicity data for honey bee (Apis mellifera) larvae exposed to thiamethoxam. All studies involved TGAI (≥99% a.i.).

Duration	Endpoints (units)	Thiamethoxam	Clothianidin equivalents	MRID/source	Classification	Comments
Acute – repeat dose	LD_{50} (µg a.i./larva/day)	0.78 (0.05 – 1.88)	0.67	49950118	Supplemental (qualitative)	Study carried out for 7 days
		>0.03	>0.03	50096607	Acceptable	Day 8 mortality endpoint based on Repeat dose on day 4 exposure/4 (>0.120/4)
	LC_{50} (µg a.i./g- diet)	11.7 (2.24-21.1) *	10.0	Tavares et al. 2015	Qualitative	Bees were Africanized.
		23	20	49950118	Supplemental (qualitative)	Value estimated based on concentrations reported by study author
		>113	>96.7	49346602	Supplemental (qualitative)	NOAEC = 35; LOAEC = 51.5 (21% mortality)
Chronic (22 d; repeat dose)	NOAEC (LOAEC) (ng a.i./g-diet)	12.5	10.7	49513601	Supplemental (qualitative)	LOAEC =25 Replicates were run at different times, composition of diet and verification of chemical concentration were not reported.
		0.028 (0.059)	0.024 (0.05)	50096607	Acceptable	None

	NOAEL (LOAEL) (µg a.i./larvae/ day) day 22 emergence					

* The study author reported a value of 0.01434 µg a.i./µL-diet (95% CI: 0.00275- 0.02594). This value was converted with an assumed density of sucrose diet (50% sugar) of 1.23 g/mL. ng a.i./µL-diet is equivalent to µg a.i./mL-diet.

Non-Apis – Open Literature Studies

In another study with bumble bees (B. *terrestris audax*), Thompson *et al*. 2014 exposed bees via sucrose to 1, 10 and 100 µg a.i./L (TGAI; 0.86, 8.6 and 86 µg c.e./L). After 4 days of continuous exposure, no significant mortality was observed in the 0.86 and 8.6 µg c.e./L test groups while 100% mortality was observed at 86 µg c.e./L (**Table 12**). Feeding was not affected at the lower test levels (*i.e.*, 0.86 and 8.6 µg c.e./L).

Table 12. Summary of open literature adult acute oral toxicity studies for non-Apis bees exposed to thiamethoxam

Test material (% a.i.)	Study Duration (Type)	Thiamethoxam (95% CI) (expressed in terms of µg c.e./bee)	Clothianidin equivalents	Test Species	Classification (Reference, MRID)
TGAI	4 d	10 µg a.i./L	8.6 µg c.e./L	*B. terrestris audax*	Qualitative (Thompson et al. 2014)

Toxicity data are also available to characterize (qualitative) effects of chronic exposures to the stingless bee larvae (*Scaptotrigona aff. depilis*) (Rosa *et al*. 2015). Effects to survival, development and morphology were observed in bees dosed with 0.000044 and 0.0044 µg a.i./larva (0.000038 and 0.0038 µg a.i./larva as clothianidin equivalents).

Tier II

This section summarizes the registrant-submitted Tier II (*i.e.*, tunnel and feeding study design) for thiamethoxam. A summary of the results and associated uncertainties is provided within the discussion of each study. The studies below, along with those outlined in the clothianidin section above indicate that exposure to thiamethoxam affected adults and brood. This conclusion is largely supported by effects seen in the colony feeding studies both sucrose and pollen based exposure test designs.

Registrant submissions - Apis

Colony Feeding Study - MRID 49757201

This registrant-submitted honey bee colony feeding study for thiamethoxam was conducted under similar parameters described for clothianidin (conducted in North Carolina, 12 test apiaries *etc*.) to assess the potential for long-term effects, including colony overwintering survival, resulting from exposure to thiamethoxam. The study was conducted June 27, 2014 to April 28, 2015. Ninety-six hives were divided according to hive strength (number of brood frames) with the strongest 8 hives assigned to Apiary A and the weakest 8 hives assigned to Apiary L (*i.e.*, the study design was stratified to account for differences in

colony strength). Within each apiary, 7 hives were randomly assigned to treatment groups where five of the colonies were provided 50% sugar solution spiked with thiamethoxam at 10.7, 21.4, 32.1, 42.8, or 86.6 µg c.e./L and two of the colonies served as controls and were provided untreated sugar solution for six weeks continuously while bees were allowed to forage freely. The 8^{th} colony at each apiary served as a monitoring hive to characterize the alternative sources of forage (pollen/nectar) for the test colonies as well as to monitor for the potential contamination with other pesticides.

Ten Colony Condition Assessments (CCAs) were conducted during the study. Two CCAs (CCA1 2) were conducted prior to feeding (*i.e.*, pre-exposure phase) to determine hive strength (number of adult and developing bees) and initial hive conditions, CCAs 3-5 were conducted during the exposure phase, CCAs 6-8 were conducted post-exposure and CCA9-10 were conducted after overwintering. Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival, were measured during the course of the study.

There are three main limitations associated with the colony feeding study which reduce the utility in this risk assessment:

Late timing of exposure that coincided with normal reductions in bee activity in preparation for overwintering;
Lower than expected performance of controls; and,
Lack of overwintering success.
Almost every parameter for evaluating life stages decreased after exposure ended and there is uncertainty whether these reductions were the result of the late time of the year when the study was initiated or whether the effects were the result of treatments. The natural process of colonies reducing their size/activity in preparation for winter contributed to high variability at the later CCAs. Many of the treatment hives performed similarly to the control, especially after exposure ended. While this could be indicative of a lack of treatment effects, variability limited the extent to which treatment effects could be detected.

Control colony loss after overwintering (79%) also adds uncertainty when considering the results of individual measurements. Because so few control hives survived overwintering (potentially due to poor food stores) and performed similar to the treatment hives during exposure the results have limited utility in evaluating colony-level effects after overwintering. The study is useful for characterizing pre-overwintering effects.

There were significant reductions ($p<0.05$) relative to controls in multiple endpoints over several CCAs in colonies exposed to 86.6 µg c.e./L. In addition, numbers of larvae, pupae, pollen stores and adults declined in the 42.8 µg c.e./L group shortly after exposure. There were statistically significant ($p<0.05$) decreases in pollen stores at CCA5, CCA7 in colonies exposed to 21.4 µg c.e/L, and there were statistically significant decreases in the number of pupae at CCA5 in colonies exposed to 32.1 µg c.e/L. At the lowest test level, i.e., 10.7 µg c.e/L, no significant reductions were noted ($p<0.05$) in any of the parameters tested. There were marginally significant ($0.05<p<0.1$) reductions in numbers of eggs at CCA6 and numbers of cells containing honey at CCA5. Based on the limitations of this study, a NOAEC derived from this study is considered uncertain. There is uncertainty in whether this value is conservative based on the study limitations discussed above.

Colony Feeding Study - MRID 50432101

Similar to clothianidin, this colony feeding study was conducted to address the uncertainties associated with the lack of overwintering success in the previous CFS (MRID **49757201**). The same study design (*e.g.*

dosing, similar location) was utilized as the original MRID and the details of study design are not discussed here. No elements of the study design were expected to be significantly different[1] than the first study. This study was conducted April of 2016-April 2107 with a 6-week exposure period initiated on July 5, 2016. The nominal doses of thiamethoxam were 12.5, 25, 37.5, 50, and 100 µg a.i/kg corresponded to measured concentrations (in c.e) of 10.1, 20.1, 29.0, 43.6, 81.7 µg a.i c.e./kg.

Decreases in multiple endpoints (significant reduction [$p<0.05$] relative to controls) and declining trends were observed over multiple CCAs in colonies exposed to 81.7 µg c.e./kg including significant reduction in larvae, pupae, and pollen in CCAs 3-6 for brood matrices, and CCAs 3-8 for pollen. Pollen reduction relative to controls was also statistically significant ($p<0.05$) at CCA 4 at the 43.6 µg c.e/kg treatment level. There were marginally significant ($0.05<p<0.1$) reductions in numbers of eggs at CCAs 6-8 and the number of cells containing honey at CCA6 in the 81.6 µg a.i c.e./kg treatment level. Numbers of bees and food stores were similar in numbers and trends compared to controls (*i.e.*, no significant differences noted) at the other treatment levels. There were no significant effects detected in the number of adults; however, there was high variability in the number of adult bees, particularly in the overwintering colonies in the highest treatment groups.

Overwintering survival in the control colonies was good (87.5%; 3/24 dead) with 91.7, 83.3, 100, 91.7, and 75 percent surviving colonies in the 10.1, 20.1, 29.0, 43.6, 81.7 µg a.i c.e./kg treatments, respectively. All colonies (3/12) that were dead in the highest treatment level died before overwintering. The study authors also reported the 81.7 µg c.e/kg treatment hives exhibited a significant decreased weight difference compared to the control hives.

In the 100 ppb (T5) treatment level, multiple endpoints were significantly affected at consecutive assessment times prior to overwintering. Therefore, the lowest observed adverse effect level (LOAEL) was determined to be 100 ppb (81.7 µg a.i c.e./kg) and the no observed adverse effect level (NOAEL) was determined to be 43.6 µg a.i c.e./kg, based on significant reductions in brood matrices.

Other Registrant Submissions

In addition to the colony feeding study, there are also registrant-submitted Tier II (tunnel) studies. As with clothianidin, these studies are generally considered qualitatively in the weight-of-evidence approach while noting design flaws and the limitations. In some cases, studies were conducted using protocols which had not been reviewed in advance by EPA to better ensure that the study would address specific uncertainties identified in lower-tier testing. There is a seven Tier II studies considered supplemental by the Agency (**Table 13**)

The Tier II registrant-submitted study examined two separate single foliar applications of Actara® 25 WG (active ingredient: thiamethoxam) at 0.089 lb a.i/A to honeydew melons. For Treatment 1, application was made 10 days before flowering and for Treatment 2, application was made 5 days before flowering. Each tunnel (representing one replicate) contained one hive, covered an area of 150 m^2 and was located in a single melon field. Colonies were confined to foraging on the enclosed melon plants for 8 days (exposure phase) after which time the colonies were relocated to a separate site and allowed to forage freely for 29 days (post-exposure monitoring phase). In Treatment 1, the only effect was increased mortality observed 3 days after exposure started. In Treatment 2, increased mortality (workers and pupae) occurred relative to

[1] Comments were made by EFED via protocol review for minor differences including dosing regimen (not incorporated), increased frequency of hive matrix sampling, marking queens, increased supplemental feeding, and an earlier exposure period.

the control in the subsequent days after exposure began. No biologically relevant effects were observed in behavior or brood indices, and differences were attributed to stress of bees in the tunnels.

MRIDs 50781601 and 05781602 were semi-field tests conducted on fields sown with thiamethoxam seed treated sunflowers. In MRID 50781601 three tunnels were placed over flowering sunflower (*Helianthuus annus)* plants and observed for 7 days. The study authors noted higher dead bees compared to the control on a single day in the treatment tents, but average mortality was higher in the control tents (11.8 bees/day) than the treatment tunnels (9.9) although these numbers are comparable. The overall mean number of eggs, larval, and capped stages were similar in the control and treatment groups. The authors noted a decline in eggs and larvae in both the treatment and controls which was attributed to being in the tunnel. In MRID 50781602 tunnels were placed over seeds applied at increasing rates in two different tunnels. Mortality was higher in the higher application rate tunnel (~4230 bees) compared to the lower rate (~240 bees); however, the control tunnel mortality was the highest in the untreated control (~4700 bees). There was some qualitative observation in a reduction of food stores (honey/pollen); however, no quantitative analysis was done by the study author.

MRIDs 50781603, 050781604, and 50781605 were semi-field tests conducted on oilseed rape fields. MRIDs 50781603 and -05 were conducted under foliar spray conditions, while 50781604 was conducted using treated seed. Similar to the other seed treatment studies, the study authors concluded similar levels in observations of mortality, and decline in brood in both the treatment and the control for thiamethoxam treated seeds. For the foliar application studies there were increased mortality effects noted if sprayed during bee flight, but was generally similar in control and treatment tunnels at the end of the observation periods. Additional declines in brood were attributed to stress from the tunnels and were comparable between control and treatment tunnels. No clear treatment effects (except for mortality when sprayed during bee light) were noted by the study authors.

Additionally, although several Tier II studies described earlier in **Section 4** and which are considered qualitative for their residue information were not considered valid for assessing potential effects and are listed in **Appendix 2**.

Table 13. Tier II Tunnel Thiamethoxam Studies Submitted by the Registrant

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam 25% (*Apis mellifera*)	Application to melon	Foliar Application (0.089 lb a.i./A; 0.076 lb c.e./A) (8 days in tunnel 29 days at monitoring site)	3 replicates/trt 1 replicate/ref chemical Hives placed in tunnels prior to full flowering either 10 or 5 days before full flowering Hives: six frame, queen right, 205 brood combs, 2-5 honey/pollen combs, 3-5 brood comb colonies, 8000-11000 adults.	Mortality, Colony condition, brood development (Yes)	Increased mortality in Treatment 2 (days before flowering)	Number of replicates was low (effects data) pollen and nectar residue data used from whole flowers and honeybee guts	Supplemental Bocksch 2011 (49158904)
Thiamethoxam (Actara 25 WG) (*Apis mellifera*)	1 g ai/ha (0.86 g c.e./ha) and 5 g ai/ha (4.3 g c.e./ha) applied via foliar spray to *Phacelia tanacetifolia*	27 d	Two replicate tunnels	Mortality, colony condition, foraging activity	Increased mortality in 5 g a.i./ha treatment	No residue data were collected	Supplemental 50781603
Thiamethoxam (Actara 25 WG) (*Apis mellifera*)	80 g ai/ha or 20 g ai/ha foliar spray to *Phacelia tanacetifolia*	7-10 d	One tunnel	Mortality, flight intensity, behavior	Increased mortality,	One replicate. No residues	Supplemental 50781605
Thiamethoxam (A-9567 B) (*Apis mellifera*)	Seed treatment of sunflower at 350 or 700 g	14 d	1 replicate tunnel per treatment	mortality, foraging activity, flight	No reliable differences between treatment and	Residues were not measured; number of bees	Supplemental 50781602

	a.i./100 kg seed)			activity and behavior (no)	control groups were observed.	per hive were not reported; no replication	
Thiamethoxam *(Apis mellifera)*	Seed treatment of sunflower at 339 g a.i./100 kg seed	7 d	Three tunnels	Mortality, foraging activity, flying intensity, behavior, colony condition (number of bees, brood, presence of queen)	None	Residue data not provided. Limited observation period.	Supplemental 50781601
Thiamethoxam (Cruiser 350 FS) *(Apis mellifera)*	Seed treatment of oilseed rape	28 d	Three tunnels	Mortality, flight activity, colony condition, bee brood development	None	No residues were collected	Supplemental 50781604

DRAFT

Open literature

This section summarizes the Tier II (i.e., tunnel and feeding studies) studies that were evaluated from the open literature in addition to those listed for clothianidin (Section 4.2.1) as part of the aforementioned joint review between EPA, PMRA, and CDPR. Many studies consider both thiamethoxam and clothianidin, so overall open literature studies in both sections are considered in the weight-of-evidence for both chemicals. As noted previously, all studies are determined to be of qualitative utility for characterization purposes in this assessment. The limitations discussed below generally add enough uncertainty to warrant a qualitative use of these studies in characterizing the potential for adverse effects from exposure to thiamethoxam and/or clothianidin. These limitations are considered when deciding the weight to give each study in the overall risk conclusions

Henry et al. 2012 monitored individual freely foraging honeybee homing behavior using radio frequency identification (RFID) tagging technology in four separate treatments versus control. There were varying degrees of bee familiarity with the release site, the distance from the release site to colonies, and the type of landscape. Foragers received a single sublethal oral dose of thiamethoxam (1.34 ng/20 µL sucrose) and were released (at different distances from the hives) and assessed for mortality, homing ability for 5 to 7 days post-treatment. The study provides evidence that bees treated with thiamethoxam had fewer returning to their colonies. There were significantly lower proportions of bees returning to colonies compared to the controls control when released 1 km away from either a familiar or random location; however, the variability in the study results fails to convincingly demonstrate/equate return frequency to mortality.

Kessler et al. 2015 examined forager honeybees collected at colony entrances; newly emerged adult workers were also collected from brood comb. Cohorts of 25 bees were placed in rearing boxes and five feeding tubes were provided: (1) one with deionized water; (2) two with 1M sucrose; (3) two with 1M sucrose containing a specific concentration of a neonicotinoid (either thiamethoxam or clothianidin). The number of bees alive in each cohort was counted and food consumption determined 24 h later. The total food consumption of forager honey bees was significantly reduced only when bees fed from solutions containing 100 nM (0.00107 µg/bee/day) or 1000 nM (0.0103 µg/bee/day) thiamethoxam or clothianidin (0.00108 µg/bee/day; 0.0085 µg/bee/day respectively).

Thomazoni et al. 2009 performed a greenhouse study (conducted 2006-2007) with cotton (cultivar FiberMax 933) plants in containers were sprayed with thiamethoxam at a rate of 400 L/ha (200 g a.i./ha) at the flowering stage (≈ 50 – 55 days after germination). Spraying was done at 9:00 AM at 29ºC with 68% relative humidity. The experiment consisted of randomized blocks, with six treatments (different chemicals) and four replicates per treatment. Each plot consisted of a pot containing four plants/pot and 30 adult worker honey bees (about 5-6 days old), and confined in gauze cages 98.5 × 41cm. Spraying with thiamethoxam resulted in 100% mortality at 5.5 hrs.

Table 14. Tier II Open Literature Studies for Apis involving thiamethoxam (THX).

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)

Thiamethoxam (NR)	Treated sucrose (1.34 ng/20 µl; 1.34 ng a.i/bee; 1.15 ng c.e./bee (estimated))	Single Dose (RFID tracked)	Varied degree to which the bees were familiar with the release site (Experiments 1 vs 2) the distance from the release site to colonies (Experiments 1 vs 3), or the type of landscape (Experiments 2 vs 4). Experiment 1; bees released 1 km from their colonies Experiment 2: six groups of bees released 1 km from colony at equally spaced random sites at 1-km boundary [circumference].	Lower- and upper-bound estimates of mortality based on homing frequency (Yes)	Experiments 1 and 2, 10.2% and 31.6% of treated bees failed to return to their colonies, respectively; Homing frequency was significantly ($p<0.001$) lower in treated bees that were unfamiliar with their release site compared to bees that were familiar with their release site Experiment 3 homing failure was reduced compared to Experiment 1 but was still significant	Purity of test chemical not reported, lack of information about potential exposure to other chemicals in the area. Uncertain of return results to specific colonies.	Supplemental (qualitative) Henry *et al.* 2012 (E159247)

					homing failure was significant ($p<0.029$)		
Clothianidin (NR), Thiamethoxam (NR), Imidacloprid (NR)	Treated sucrose: COD (0.07, 0.647, 5.28 and 10.3 ng/bee /24 h) corresponds to 1, 10, 100, and 1000 nM; IMI (0.064, 0.418, 3.98 and 13.9 ng/bee) corresponds to 1, 10, 100 and 1000 nM ; THX (0.105, 1.05, 10.3, 33.6 ng/bee) corresponds to 1, 10, 100, and 1000 nM).	Behavioral two-choice assays: 24 h Honey bee antennal and mouthpart assays: not stated 3. Electrophysiology experiment: 2 s	Three experiments: Behavioral two-choice assays: Honey bee antennal and mouthpart assays: 3. Electrophysiology experiment	Food consumption, survival; The feeding reflex (proboscis extension reflex, or PER); Electrophysiological recordings from taste neurons (Yes)	The authors report honeybees did not avoid concentrations occurring in the range of 1 – 100 nM and the highest concentrations of thiamethoxam and clothianidin tested (1 µM) significantly reduced their survival; Proboscis extension or retraction was not affected; Stimulation with imidacloprid thiamethoxam.	Feeding was conducted under stressful conditions.; Choice oral tests were conducted not according to any official protocol; PER and electrophysiology studies are very artificial, conclusions should be made cautiously on this type of lab based experiments where only part of a bee are examined and not the whole (*let alone* at the colony level)	Supplemental (qualitative) Kessler *et al.* 2015

					or clothianidin did not elicit spikes from any of the neurons in the galeal sensilla honeybees or spiking activity of sucrose-sensitive gustatory neurons in the		
Thiamethoxam (NR). And other insecticides	Spray 200 g a.i./ha (thiamethoxam) (171 g c.e./ha)	Greenhouse study conducted on cotton, November 2006 - January 2007 in Brazil	Randomized block with six treatments and four replicates per treatment Number of cotton plants maintained in a pot per plot: 4. Number of A. *mellifera* adult workers per pot per gauze cage (98.5 x 42 cm): 30.	Mortality	Spraying with thiamethoxam lethal for A. *mellifera* causing 100% mortality 330 minutes	Effects on the behavior of the honey bees after treatment application were not documented. No access to the raw data to confirm statistical analyses	Supplemental (qualitative) Thomazoni *et al*. 2009

Tier II studies - Non-Apis

There were 3 Tier II registrant-submitted studies and 5 open literature studies considered to characterize the colony-level effects on bumble bees (*Bombus* spp.) to thiamethoxam (**Table 4.29**). As with the higher-tier *Apis* open literature studies, exposure duration, concentrations tested, and endpoints assessed varied across these studies, and many of the same limitations are noted.

Some *Bombus* studies are conducted with microcolonies. Microcolonies are queen-less units of a few worker bumble bees where one individual eventually becomes dominant and starts laying unfertilized eggs (*i.e.*, males).

Registrant Submitted Studies

Two studies submitted by Reber (PMRA#s 2364898 and 2364900) looked at effects on bumble bee colonies (B. *terrestris*) in tents following drip or foliar application of thiamethoxam. Colonies were placed in tents immediately after application (Actara™ 25 WG foliar rate 100 g a.i./A; drip irrigation rate – 150 g a.i./A). While overall there were no differences between treated and control groups for foraging activity or behavior following drip irrigation, there were significant ($p<0.05$) reductions in bees foraging activity in the treated group following foliar applications. The foliar application noted affected bumble bees exhibited irritation, erratic motions, were paralyzed and in a dorsal position before dying or that affected bumble bees were hanging on the tomato leaves and died afterwards.

A study submitted by Balluf (2001) looked at foliar applications of 0.1 kg a.i./ha with split applications and different time intervals (21/14 and 9/2 days before exposure). The study did not find any effects of either treatment on mortality, foraging activity, food consumption, or growth of bumble bee colonies.

Open Literature

Mommaerts et al. (2010) examined the effects of Actara™ 25 (25% TGAI) to bumblebees (Bombus terrestris) from oral exposure in sugar water for 11 weeks, under two different conditions in the laboratory, i.e., considering and not considering foraging behavior. Worker bumblebees (four artificial nests each with 5 bumblebee workers per treatment) were exposed to thiamethoxam at concentrations ranging from ranging from 0.01 to 100 ppm via ingestion of spiked sugar water; bees were evaluated for survival, nest development and reproduction (drones produced), and foraging behavior. Increased (compared to control) worker mortality was noted in the thiamethoxam treated groups, and the nests exposed to 100, 10, 1.0 and 0.5 ppm thiamethoxam showed a total loss of reproduction, while at 0.1 ppm the numbers of drones were significantly ($p < 0.05$) lower than the controls with no difference observed at 10 ppm. Some of the limitations of the study included a lack of quantification of test material, potential stress from the study design, and a lack of information on the control group.

Alacrcon et al. 2005 examined effects to bumble bee (Bombus terrestris L.) colonies (30 recently born or just born workers, a queen, and pupae) from thiamethoxam (Actara®) applied through drip irrigation at a rate of 200 g a.i./ha and as a split application (100 g a.i/ha) of the same total rate, and compared to imidacloprid (toxic standard) applied as foliar at 15 g a.i./ha. Treatment took place 2 days after the colonies were introduced into greenhouse containing the treated tomato plants (hives were closed and opened after application). Two consecutive trials (3/9/14-4/26/14 and 4/27/14-6/7/14) were made on the same tomato crop. The duration of each trial was 6 weeks. There were no effects for mortality, foraging activity, or pollination rates in both trials between the treated and control plots. Visual evaluation of the data suggests effects (lower adult, larvae and pupae counts) in the treated hives compared to the control

with more pronounced effects seen from one drip application of 200 g a.i./ha compared to the two drip applications of 100 g a.i./ha each. However, there was poor control performance (especially in the second trials) relative to the reference toxicant adding considerable uncertainty to the results.

Sechser and Freuler 2003 (MRID 49579001) examined the effects of a thiamethoxam drip irrigation application scenario to adult bumble bees (B. terrestris) and brood. Tomato plants within greenhouses (1 replicate) or tunnels (1 replicate) were treated with thiamethoxam at rates from 150 to 161 g a.i/ha (0.13 to 0.14 lb a.i/A). A single bumble bee colony was placed inside a greenhouse (1) or two colonies in a tunnel (1) and bees freely foraged on tomato plants as well as supplemental bumble bee food (nectar) and pollen that were provided within the greenhouses. After 13 to 35 days of exposure, there were no differences between the hives exposed to thiamethoxam and the negative controls. However, limitations included no replication within treatment groups, high variability in the results, and exposure uncertainties (rate of uptake into pollen and nectar and/or lack of measurement to confirm exposure).

Elston et al. 2013 (MRID 49579002) examined the effects on nest building or brood production from dietary exposure of thiamethoxam (or propiconazole) in B. terrestris microcolonies. Bees were exposed for 28 days to thiamethoxam concentrations of 1 or 10 µg/kg in honey water and pollen paste. For thiamethoxam, both dietary exposures reduced consumption of honey-water and the number of wax cells ("honey pots"). At the 10 µg/kg treatment, nest building initiation was delayed, fewer eggs were laid, and no larvae were produced.

Laycock et al. 2014 used microcolonies of B. terrestris L. Workers were exposed to a wide range of dietary concentrations up to 98 µg/kg in syrup for 17 days while also feeding clean pollen. Bumblebee workers survived fewer days relative to controls when presented with syrup at 98 µg/kg, while production of brood (eggs and larvae) and consumption of syrup and pollen in microcolonies were significantly ($p<0.05$) reduced by thiamethoxam only at the two highest concentrations (i.e., 39 and 98 µg kg-1).

Stanley et al. 2015 investigated how exposure to thiamethoxam could affect the ability of bumblebees to pollinate apple trees. Colonies were pre-exposed to thiamethoxam at 0, 2.4 or 10 ppb in artificial sugar water for a period of 13 days (8 colonies per treatment). Afterward, treated colonies were brought to the field, allowed access untreated apple trees, and observations were collected at both the individual- and colony-level behavior. The study authors reported that in the 10 ppb treatment there were lower visitation rates to flowers and lower numbers of bees carrying pollen compared to controls ($p = 0.05$, and 0.008 respectively), in addition to suggesting that thiamethoxam exposure altered how bees behave on flowers.

Stanley and Raine 2017 investigated colony growth of bumblebees by exposing *Bombus terrestris* colonies (via treated sucrose for 27 days) to 2 levels (2.4 and 10 ppb) over 4 weeks and observed them in the lab. The study author's reported no impact of insecticide exposure on colony weight gain, or the number or mass of sexuals produced, although colonies exposed to 2.4 ppb thiamethoxam produced fewer males (this difference was not statistically significant) that were larger than those in the control or 10 ppb exposure group.

Stanley et al. 2016, investigated the impact of chronic exposure (5–43 days) to field-realistic levels of a neonicotinoid insecticide (24 ppb thiamethoxam) on foraging ability, homing success and colony size using radio frequency identification (RFID) technology in free-flying bumblebee colonies. Pesticide treatment colonies received a feeder of 40% sucrose solution in the external chamber that contained approximately 2.4 ppb thiamethoxam. The author's reported individual foragers from pesticide-exposed colonies carried out longer foraging bouts ($P<0.05$) than untreated controls (68 vs. 55 min). Pesticide-exposed bees also

brought back pollen less frequently ($P<0.05$) than controls indicating reduced foraging performance, while no overall impacts to colony size were found relative to the control.

Baron et al. 2017 took wild caught bumblebee queens from 4 species to examine effects of field realistic exposure to thiamethoxam (1.9-5.3 ppb). Queens were fed for 14 days, and observed for 14 days after for signs of mortality, waxing behavior and egg laying as well as ovary development. The authors reported exposure to 5.3 ppb of thiamethoxam resulted in feeding reduction in 2 species ($p<0.05$). No impacts were reported to egg laying; however, it was noted a low number of queens laid eggs during the experiment.

Table 15. Tier II studies characterizing the toxicity of thiamethoxam to non-Apis colonies.

Open Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] ([all comparisons made relative to the study's control])	Limitations[2]	Classification Citation (MRID Number)
Registrant Studies							
Thiamethoxam Actara 25 WG (25%) (*Bombus terrestris* L)	Foliar application (1 hand sprayer 100 g a.i./ha; 85.6 g c.e./ha) Fed 50% sugar solution inside the hive ad libitum	Single Foliar Application (28 days 6/18/98-7/16/98)	Tomatoes (10-12 week old plants, first flower stage), 2 plants per pot (35 cm diameter, 20 L volume) and 16 pots per tent. bumble bee hives placed in tent immediately following application. three rep/trt	Pollination activity, behavior, mortality, and vitality (1, 2, 4, 7 days post trt then 2-3 days until endo of exposure.)	Reduced pollination activity (2 weeks after exposure) Effects on behavior (irritation, uncontrollable motions, paralysis). High mortality. Eggs and larvae could not be monitored since there were no larvae or eggs present at study termination in any treatment group.	Initial number of bees in the hive and/or an estimate of the total number of bees in a hive throughout the experimental duration is not reported The hives were within the same treatment area, which represent repeated measures and not true replicates. It is uncertain what the residues were in the pollen	Supplemental Reber 1999a PMRA 2364900

Thiamethoxam Actara 25 WG (25%)	Foliar application (hand sprayer 150 g a.i./ha; 128 g c.e./ha)	Single Foliar Application (28 days 6/18/98-7/16/98)	Tomatoes (10-12 week old plants, first flower stage),	Pollination activity, behavior, mortality, and vitality (1, 2, 4, 7	No effects on pollination activity of bumble bees.	The hives were within the same treatment area, which represent repeated	Supplemental Reber 1999b

(*Bombus terrestris* L)	Fed 50% sugar solution inside the hive ad libitum		2 plants per pot (35 cm diameter, 20 L volume) and 16 pots per tent. bumble bee hives placed in tent 1 day prior to application. three rep/trt	days post trt then 2-3 days until endo of exposure.)	No effects on behavior and repellency. Overall, the mortality was high in all test groups Eggs and larvae could not be monitored since there were no larvae or eggs present at study termination in any	measures and not true replicates. It is uncertain what the residues were in the pollen	PMRA 2364898
Thiamethoxam Actara 25 WG (25%) (*Bombus terrestris* L)	Foliar application (2 X hand sprayer 100 g a.i./ha; 85.6 g c.e./ha) Fed 50% sugar solution inside the hive ad libitum	TRT 1: Tomatoes, BBCH growth stage was 21 (first primary apical shoot visible) to 29 (nine or more apical shoot visible) TRT 2: 51 (first inflorescence visible) to 62 (second inflorescence first flowers to open) for treatment 2 scenario	4 reps/trt Initiated 10/31/00 ad 11/1/00 TRT 1: Application 21 and 14 days before hives TRT 2: 9 and 2 days before hives	Food consumption, Colony weight, Mortality, Foraging activity, Brood	No effect of either treatment scenario on mortality, pollination, consumption of sugar, growth of colonies or brood.	It is uncertain what the residues were in the pollen Uncertain if control group mortality is high	Supplemental Balluf 2001 PMRA 2364997
Open Literature							
Thiamethoxam Actara 25 WG (25%) *Bombus terrestris* L	Treated sugar water (100 µg a.i./L; 85.6 µg c.e./L)	4 colonies with 5 workers each housed in cages for 11-week exposure	Bees were exposed orally to pesticides via treated sugar water in box plain sugar water and no worker mortality was observed after	mortality, drone production -- (Yes)	85% of worker toxicity were observed, significant sublethal effects	There was no analytical confirmation of thiamethoxam in the treatment solutions	Qualitative Mommaerts, 2010 48151502

			11 weeks.		(p<0.05) as the drone production was very low	Control performance was not reported	
Thiamethoxam Actara 25 WG (25%) *Bombus terrestris* L	Greenhouse 1 app Drip irrigation (200 g a.i./ha; 171 g c.e./ha) Split app drip irrigation (100 g a.i./ha; 85.6 g c.e./ha) Fed 50% sugar solution inside the hive ad libitum	Split application (100 g a.i/ha 7 days apart 3/11, 3/18/04 - 4/26/04). Single application (200 g a.i./ha - 3/11/04 - 4/26/04).	1280 m^2 with four plots each measuring 320 m^2 (40 x 8 m) were used	Pollination, Mortality, Food consumption, Brood production, Colony strength	Single app: Significant (p<0.1) mortality Split app: No sig differences observed	Control hives performed worse than reference toxicant Statistical analysis was conducted for the pollination rate but there was no mention on what method of statistical analysis was used. Raw data was not included in the study.	Qualitative Alarcon *et al.* 2005
Thiamethoxam (TGAI) *Bombus terrestris* L	Artificial nectar solution and pollen paste (1, 10 µg a.i./kg; 0.86, 8.6 µg c.e./kg;)	28-day exposure	4 trt (including neg and solvent control) 10 reps replicates in each:	Worker mortality, nest- building activity, egg laying, and bee behavior	reduction in nectar consumption and storage Delayed colony development Fewer eggs/larvae/was cells (10 µg/kg) and reduced nest building	No verification of test substance in pollen or nectar No Raw data to confirm statistical conclusions	Qualitative Elston *et al.* 2013 49579002
Thiamethoxam NR	Treated sugar solution with untreated pollen	17-day exposure	Queenless microcolonies of *Bombus terrestris* L.	Worker mortality, wax covered egg cells, brood	Reduced survival (98) and reduced brood production and food	Bumblebees may forage on mass-flowering crops	Qualitative Laycock *et al.* 2014
Unspecified (% unspecified)	2.4 ppb ai (nominal) (2.1 ppb c.e.)	43 days	8 colonies (4/level) located in lab with	*Foraging activity:*	Increased foraging trip duration per	Single treatment level	Stanley et al (2016)

Bombus terrestris	1.74-2.34 ppb ai (1.49-2.00 ppb c.e.) (range measured in 3 samples) Sucrose solution and acetone	(*foraging*: starting day 5 of exposure to ca. exposure termination; *homing*: starting after 2 weeks of exposure to ca. exposure termination; *colony growth*: test initiation to exposure termination	unrestricted access to forage on flowers outside Exposed in lab to spiked sucrose solution (replenished 3 days/week) One treatment level and one solvent control	# drifters/colony, # days foraged/bee, # foraging bouts/day/bee, # visits/day/bee, foraging trip duration/day/bee, # foragers/colony, # foragers retuning to colony, proportion of bees carrying pollen/colony *Homing ability:* Proportion of bees returned from 1 or 2 km away/colony, time taken to return 1 or 2 km/bee, proportion of bees that returned overall/colony, time taken to return overall/bee *Colony growth:* # callows emerged/colony, # dead bees/colony, # dead bees that did not return/colony, colony size, body length/bee -- (Yes)	bee per day and proportion of bees that returned when released 1 km from their nest per colony Decreased proportion of bees that returned carrying pollen per colony No statistically significant differences on colony growth or additional measured variables related to foraging activity and homing ability	No negative or positive controls Unclear how much sucrose was consumed and therefore unknown actual exposure (per day and cumulative) Analytical measurement of only 3 samples to confirm exposure concentration Potential exposure to other pesticides in surrounding landscape, which is multi-purpose use. No screen of pollen returned by bees for potential exposure to other pesticides Single trial Foraging activity and homing ability analyses represented a range in duration of exposure	

Bombus terrestris L	(dosages s (µg/kg thiamethoxam: 98.43, 39.37, 15.75, 6.30, 2.52, 1.01, 0.40, 0.16, 0.06)		two replicate trials between October and December 2012. Each trial comprised 50 microcolonies	production (eggs/larvae)	consumption (39, 98)	throughout their bloom (> 17 d exposure) Multiple sources of bees used No Raw data to confirm statistical conclusions	
Thiamethoxam (NR) *Bombus terrestris*	Residue: P acetifolia: 40 g a.i./ha (34 g c.e./ha) Foliar tomatoes: 100g a.i./ha (86 g c.e./ha) ;40 g a.i./ha (34 g c.e./ha) Single drench: 150 g a.i./ha (128 g c.e./ha) Tunnel: 172 g a.i/ha (147 g c.e./ha); 161.1 g a.i./ha (138 g c.e./ha)	Foliar application P. acetifolia (contact) – 3 weeks Foliar application tomatoes 28 then 24 days after exposure Drench application tomatoes 28 days Tunnel studies 28 days;35 days	See review. Multiple study designs present.	Mortality, pollination activity, foraging activity	Foliar application P. acetifolia – mortality 92% compared to controls, drops to ~68% after 7 days in the second series. (Foliar application tomatoes mortality 93% compared to 58 % controls, with 68% at 14 days in the second series Drench application tomatoes – comparable mortality to control Tunnel studies 28 days;35 days	•For the plastic tunnel studies, the effects of the untreated control for Trial 1 were not Growing conditions of the tomato plants were not mentioned. There was no mention on when the studies were conducted. No information on the conditions during the conduct of the study for the semi-field conditions and plastic tunnel studies.	Qualitative Sechser *et al.* 2002

						No mention of other sources of pollen. No access to the raw data to confirm statistical analyses.	
Thaimethoxam (B. terrestris)	Tomato plants within greenhouses (1 replicate) or tunnels (1 replicate) were treated with thiamethoxam at rates from 150 to 161 g a.i/ha (0.13 to 0.14 lb a.i/A; 0.11 lb c.e./A to 0.12 lb c.e./A).	13-35 d	Bees kept in tunnels or greenhouses	Number of larvae, adults, amount of pollen and nectar	None	no replication within treatment groups, high variability in the results, and exposure uncertainties (rate of uptake into pollen and nectar and/or lack of measurement to confirm exposure)	Supplemental Sechser and Freuler 2003 (MRID 49579001)
Analytical standard (% unspecified) *Bombus terrestris*	2.4 and 10 ppb ai (nominal, v/v) (2.1 and 8.6 ppb c.e.) Sucrose solution and acetone	26-27 days (39-41 days; bees monitored an additional 13-14 days after exposure period; colony weight measured weekly and other measures at test termination)	24 mature colonies (8/level) located in lab Each day, three colonies (1/level) began treatment for 8 consecutive days Exposed in lab to spiked sucrose solution (replenished daily) and an equal amount of untreated pollen Two treatment levels and one solvent control	Colony weight # bees (worker, male, queen) Dry weight/bee (worker, male, queen) Total biomass/colony (workers, males, queens) --	None	Two treatment levels No negative or positive controls "*All sucrose solutions were actively consumed*". It is unclear if this indicates that each colony consumed the entire sucrose solution each day. No analytical measurements to	Stanley and Raine (2017)

				(Yes)		confirm exposure concentration Did not screen pollen for pesticides Single trial	
Thiamethoxam (NR) *Bombus terrestris* audax	Treated Sucrose 2.3 and 10 ppb a.i. (2.0 and 8.6 ppb c.e.)	12-15 days (60 mins for 8 days and at test termination)	24 colonies (avg 99 workers) 8/trt exposed to thiamethoxam Brought to apple orchard and observed for pollination services	Entry/exit from colony boxes, bees carrying pollen, flower visitation rate	In 10 ppb lower visitation rates to flowers and lower number of bees carrying pollen	Conservative exposure scenario (not representative of field-level) Orchard details left out No analytical verification text concentrations	Qualitative Stanley *et al.* 2014
Thiamethoxam analytical standard (% unspecified) *B. terrestris, B. lucorum, B. pratorum, B. pascuorum*	Solvent control, 1.87, 5.32 ppb ai (measured) (1.60 and 4.55 ppb c.e.) *Slight contamination in control (0.063 ppb ai)	2 weeks (4 weeks total: 2 exposure followed by 2 post- exposure)	Spring-caught wild queens from a site with known pesticide use 39-50 bees per level; however, fewer were used for analysis: some bees escaped and bees were excluded if found to be infected with parasites. B. lucorum (5-12 bees used in analysis); B. pascuorum (15-17 bees); B. pratorum (15-22 bees); B. terrestris (32-35 bees)	Oocyte length, feeding, survival, waxing behavior	B. terrestris: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced length of terminal oocytes No effects on feeding, survival, or waxing behavior B. lucorum: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced length of terminal oocytes	No negative control (solvent control only) or positive control	Baron et al (2017)

			Spiked sucrose syrup solution + pesticide-free commercial pollen pellets)		No effects on feeding, survival, or waxing behavior B. pratorum: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced feeding and length of terminal oocytes No effects on survival, or waxing behavior B. pascuorum: NOAEC = 1.87 ppb ai LOAEC = 5.32 ppb ai based on reduced feeding and length of terminal oocytes No effects on survival, or waxing behavior		

Tier III

The following section describes the Tier III studies either submitted by the registrant or identified in the open literature. Studies considered below and with those listed for clothianidin are considered when evaluating the potential for adverse effects from exposure to thiamethoxam.

Registrant submissions

There are thirteen registrant-submitted Tier III field studies on various crops submitted (**Table 16**). These studies are generally considered to be of limited utility in risk assessment based on the strength of their design and resulting effects. Five honey bee field studies, which are classified as supplemental, including Szentes 2001a (46163102), Szentes 2001b (46163103c), Frana 2003 (46241601) Balluf 2003 (46163103a), Schur 2001 (46163103b) were conducted in Hungary, Argentina, Spain, and Italy and examined the effects of thiamethoxam-treated sunflower seeds on honey bee colonies: mortality, foraging behavior, overall behavior, and colony strength. Two studies were conducted with CRUISER® 70 WS (70%) and three studies were conducted with CRUISER® 350 FS (30-35%). Overall, transient effects were seen on mortality mostly after application with no treatment-related effects detected on brood or adult foraging. Two submitted studies were conducted in France using oilseed rape over 4 years (Hecht-Rost 2009 48053301 and Hecht-Rost 2009 48053302) with no statistically significant effects detected on brood development, and only statistically significant ($p<0.05$) effects noted on honeybee mortality dependent on specific years in the multi-year studies. A study (Mayer 1998 44714929) was conducted on apple orchards in 1998 and again noted no effects relative to control plots.

Additional studies were also submitted on pome fruits, oil-seed rape, and melon. These studies exposed honey bees to treated orchard crops: 44714929, 5076602, and 50766604 (apples), and 48584701 (pears). No effects were detected on bee mortality, flight activity, behavior and brood from pre-bloom or post bloom (via available residues) applications to apple trees treated with 100 to 200 g a.i/ha. No effects were detected on bee mortality from pre-bloom application to peach at 62.5 g a.i/ha (with declining residues over time) when applied 15 days before bloom but higher mortality and reduced foraging activity from pre-bloom application were identified when applied 7 days before bloom. No effects were detected on bee mortality or foraging activity from pre-bloom application to pear at 95 g a.i/ha 5, 8 or 11 days before bloom. However, statistically significant higher mortality (relative to controls) was observed from pre-bloom applications at 1 and 3 days before bloom.

Common limitations noted in these studies include uncertainty of exposure and the origin of the pollen and nectar brought back to the hives, high variability in the data collected (including in control hives), and lack of suitable replication or pseudo-replication. Additionally, close proximity between control, treatment, and both control/treatment plots may have resulted in cross foraging, and intrinsic to field studies, availability of alternate forage which are also uncertainties.

Table 16. Thiamethoxam Tier III Registrant-submitted Studies for Apis.

Test Substance Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects2 (all comparisons made relative to the study's control)	Limitations	Classification Citation (MRID Number)
Thiamethoxam 25.2% (*Apis mellifera*)	Application to apples	Application 4-7 days prior to bloom at 0.04 lb c.e./A (16 days Mortality – daily all days Foraging – 8/16-8/23) Hive strength 8/4, 8/24	Apple (Malus sp.) orchard located in the Yakima Valley of South Central Washington State. Each plot (replicate) measured between 0.24 and 0.40 ha Treatment and control plots were separated by at least 305 m (1000 ft.). 3 colonies per treatment, distributed in the orchards on day 0 after the application.	Mortality, foraging, brood development (Yes)	No abnormal effects on mortality/foraging or hive strength found	# Bees/Colony not provided Uncertainties in the pooled control origins used for comparisons by the study author	Supplemental Mayer 1998 (44714929)
Thiamethoxam 35% (*Apis mellifera* carnaca)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.016 lbs a.i/A Observations: Mortality/Foragin/behavior – daily all days Hive strength 6/22/00, 7/4/00 (12 days)	Fields located in Tolna, Hungary 15 hives/trt Plots were 63.2 h (C); 155.0 Ha (trt) with 8000 m between	Mortality, foraging, brood development, residues honey/nectar/pollen and flower heads (No)	No treatment effects mortality, foraging, behavior, or brood development observed	No replication (1 control/ treated field) Lack of pollen analysis to confirm foraging on treated field	Supplemental Szentes 2001a (46163102)

Thiamethoxam 30% (*Apis mellifera* L.)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.02 lbs c.e/A Observations: Mortality/Foraging/behavior – day 2-12 Hive strength day 0 and day 13 (12 days)	Fields located in Hungary Control fields were 4.5/15 ha; treated field was 4.5 ha. 15 hives/trt	Mortality, foraging, brood development, residues honey/nectar/pollen and flower heads (No)	Mortality higher up to 7 days post treatment. No apparent treatment effects on behavior, brood development, colony strength	No replication (2 control/ 1 treated field) Lack of pollen analysis to confirm foraging on treated field Attractive melon fields were close 500-1500 m to treatments/control Data were not reported for each hive.	Supplemental Szentes 2001b (46163103c)
Thiamethoxam 34.8% (*Apis mellifera* L.)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.006 lbs c.e/A Observations 2/21/01-4/11/01: Mortality/Foraging/behavior – daily Hive strength day 3, 13, 49 days after treatment (9 days mortality/behavior 49 days brood)	Fields (>2km apart) located in Santa Fe, Argentina, 20,448-22050 m2 6 hives/trt	Mortality, foraging, brood development, residues honey/nectar/pollen and flower heads (No)	No treatment effects on honey bee mortality, foraging, behavior, and brood development	Thiamethoxam were detected in the control pollen No replication (1 control/treated [and 1 reference] field)	Supplemental Frana 2003 (46241601)

Thiamethoxam-282 g/L Fludioxonil-8.00 g/L Mefenoxam-33.4 g/L (*Apis mellifera* L)	Seed Treated (A9807C) Oilseed Rape (0.03 lb c.e./A maximum)	4-year study. Observations during first 9 days of exposure and thereafter 2-3 days through the end of exposure (21 days) Brood development assessed at start of exposure and approx. 7 days thereafter	Alsace France at different locations in each year. 2-3 ha, fields separated by 1.8 to 7.5 km. with 6 colonies per control/treatment fields. colonies were set-up and maintained at the exposure location until the end of the flowering period. Colonies were then relocated to their monitoring and over-wintering location (forest near Hegency, France).	Mortality, foraging activity, behavior of the bees daily during Brood development	In 2006, control mortality > treatment mortality (t = 3.66, p = 0.005). No other significant treatment related effects	Lower application rate (and variable over the 4 years) than the highest labeled rate. Different control seed treatments in different years and in different places Limited residue/pollen analysis (LOD not reported)	Supplemental Hecht-Rost 2009 48053302
Thiamethoxam-Cruiser WS 70 (70%) *Apis mellifera* L)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.02 lbs c.e/A Mortality/Foraging/behavior – daily (16 days) Brood assessments day 1, 10, 19, and 48 days after treatment (16 days mortality/behavior 48 days brood)	Fields located in SW Spain Fields were ~40000m2 and 3.7 miles apart 6 hives/trt Reference chemical trt imidacloprid	Mortality, foraging activity, behavior of the bees, brood development as well as residues in sunflower blossoms, honey, pollen, bee honey, stomach	Increased mortality and flight intensity 5, 6, and 7 days after treatment No treatment effects on behavior, colony strength, the queen, or brood development	No replication (1 control/treated [and 1 reference] field); Short observation period	Supplemental Balluf 2003 (46163103a)

Thiamethoxam-Cruiser WS 70 (70%) *Apis mellifera* L)	Thiamethoxam Treated Sunflower (*Helianthus annuus*), seed	Application 0.02 lbs c.e./A Mortality/Foraging/behavior – daily (10 days) Brood assessments at 2, 9, and 40 days after treatment (10 days mortality/behavior 49 days brood)	Fields located in Central Italy, Fields were ~20000m2 and > 1.2 miles apart 6 hives/trt Reference chemical trt imidacloprid	Mortality, foraging activity, behavior of the bees, brood development as well as residues in sunflower blossoms, honey, pollen, bee honey, stomach	Increased mortality days 7 and 8 and flight intensity day 8 significant reduction in the number of capped cells No treatment effects on behavior, colony strength, the queen, or most of brood development.	No replication (1 control/treated [and 1 reference] field); residues of Thiamethoxam were detected in one control pollen sample Short observation period	Supplemental Schur 2001 (46163103b)
Actara 25 WG (25.0%) *Apis Mellifera*	Pre-bloom application to pears	1 pre-bloom application at different times (6 TRTS) at 0.07 lb c.e./A observed for 14 days	1 control and 6 treatments. Hives placed in plots 10 acres with 40 trees and 10 /replicate Application 11, 8, 5, 3, and 1 day before bloom	Mortality, foraging, colony strength	Statistical differences in mortality when sprayed 3 and 1 days prior to bloom; author notes there was high variability regardless of treatment group	One plot per treatment No residue analysis was conducted Treatment plots were next to each other (potential cross contamination)	Supplemental 48584701
Actara 25 WG (25.0%) *Apis Mellifera*	Drip irrigation to honeydew melon plants	1 application at 0.15 l.b. c.e./A observed for 14 days	Two treatment (T1/T2) fields, one control, one reference 2 km apart 4 hives/field T1 – treated 1 wk after planting T2 – treated during bloom	Mortality, foraging activity, colony condition, hive weight, behavior (Yes)	No significant effects on colonies noted. Transient mortality effects in treatments on Day 2 after application	T1 and T2 were different application scenarios as well as reference (foliar). No residues were taken to confirm exposure/no melon pollen was found in pollen traps	Supplemental 50766601

Actara 25 WG (25.0%) *Apis Mellifera*	Post-bloom application to apples (*Malus domesticus*)	2 post bloom apps 7 days apart @ 0.08 lb c.e./A 21 days in field observed for 8 weeks post application	Application was made during bee flight after fruit fall and when frit was 10-20mm 1 trt and 1 control field 5 km apart 4 hives/field	Mortality, foraging activity, colony condition, hive weight, behavior (Yes)	No significant effects on colonies noted.	Although used in accordance with the label directions, post bloom applications mostly limits exposure to contact only in during the 21 days in the field	Supplemental 50766602
Actara 25 WG (25.0%) *Apis Mellifera*	Post-bloom application to apples (Gala, Elstar)	1 post bloom application at 0.08 lb c.e./A 14 days (mortality behavior) in the field and observed for 29 days (colony condition)	One treated field (no control) with 4 hives Sprayed during bee flight	Mortality, foraging activity, colony condition (Yes)	. No significant effects on colonies noted. No effects on mortality during exposure.	Although used in accordance with the label directions, post bloom applications mostly limit exposure to contact only. Adjacent fields had also flowered, so forage distance as likely high (and would also limit drift exposure). No control for comparison limits interpretation of results	Supplemental 50766604

Thiamethoxam WS (70.1) *Apis Mellifera*	Seed treatment to oilseed rape (*Brassica napus*)	Oilseed was sown at 0.02 lb c.e./A 11 days for mortality, behavior) and observed for 46 days (colony condition)	One control and one treated field w/6 hives/field during full flowering	Mortality, foraging activity, colony condition, hive weight, behavior (Yes)	No significant effects on colonies noted. Increased morality during exposure period Decreased hive weight	Mortality was higher in control than treatment for one observation period (attributed to robbing by study author). No residue analysis to confirm magnitude of exposure (pollen analysis confirmed foraging on treated field)	Supplemental 50766603

Open literature

Two additional field-level studies were evaluated from the open literature (Table 4.33). Both were determined to be qualitative in nature, as it was uncertain if the test designs were robust enough to evaluate treatment effects.

Thompson et al. 2016 used RFID tags on free-foraging honeybees to evaluate survival and foraging/homing activity at varying distances from either untreated winter oilseed rape or winter oilseed rape grown from seed treated with thiamethoxam (as Cruiser™ OSR). There were no obvious trends (the data were not amenable to statistical analysis) between the control and treated groups across the three tested distances from the fields; however, visual observation indicates colonies located within 1 km from treated fields may be more likely to be impacted (decreased mean foragers life span, total flying days, mean trip durations and mean total flying time per bee for foragers). Pollen was identified to family level, and there was uncertainty as to the actual proportion of oilseed rape pollen utilized by either the control or the treatment colonies, which may have influenced the ability of the study to detect treatment effects.

Tremolada et al. 2010 examined the effects on hives from exposure to residues from sowing operations with Cruiser®- and Celest® xl- treated corn seeds. The study indicated effects on honeybee mortality, during planting while control hives located 200 m away from the test site and protected by a vegetation barrier showed no apparent effect on mortality. The mortality observed in the control hives and the treatment hives were similar before sowing. The control hive mortality did not differ during the day of sowing; however, mortality in the treatment hives increated to >40 dead bees/day. Shortly after the sowing period, bee mortality in the exposure hives decreased back to about 10 bees/day. However, except for the day of sowing, the control hives had higher mortality on all other days compared to the treatment hives. There was also some indication of decreased foraging after planting after a (visually observed) decrease in number of foragers (9.3) compared to controls (23) was observed; however, the number of foragers recovered to pre-planting numbers. This study was comparatively short and measured more individual effects (from exposure during sowing operations) rather than brood development/colony effects (from foraging on treated corn pollen).

Table 17. Thiamethoxam Tier III Open Literature Studies for Apis

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed – (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam (Cruiser®; 350 g L^{-1}) 47.6% Apis mellifera L	Seed treated winter oilseed rape (0.020 mg thiamethoxam/seed)	5-week exposure during flowering (16 May–20 June 2013). Foraging observations collected from 16 May - 20 June 2013; one disease	Frequency identification transponders (RFID tags) on free-flying honeybees (Apis mellifera L). 36 colonies used with 12 colonies per study field (2 control fields and 1 treated field), 3 apiary sites Study hives were located at the field edge (on-field site), approximately 500m (0.5 km site) or 1000m (1.0 km site) from the fields of oilseed rape.	lifespan and foraging/homing activity	No obvious trends reported between the control and treated groups across the three tested distances from the fields Results do suggest foragers farther away from treated field were affected (homing behavior, lifespan, and reduced foraging).	Lack of replication (1 treated field and 2 controls) Colonies placed at 20% bloom (treatment). Additional forage was possible diluting exposure Residue analysis unclear if robust enough to capture variation in a treated field	Qualitative Thompson et al. 2016
Thiamethoxam (Cruiser®; 350 g L^{-1}) Apis mellifera L	Treated corn seed (7.35 g a.i./ha)	Sowing on 6/22/2008 (6 days of observation)	2 hives/treatments 4 hives/control agricultural farm in the south-east of Milan, Italy control hives placed inside the farm garden (approximately 200 m away from the treated fields). The exposure hives were located at	Direct mortality Foraging activity (Yes)	Greater mortality in the exposure hives the day of sowing – decreasing Shortly after significant effect of treatment (p=0.024) and time (p=0.020) on mortality.	monitoring of honey bee mortality and foraging activity was limited due to weather conditions. Study duration short – Pollen/nectar carried by the bees back to the	Qualitative Tremolada et al. 2010

			the field hedge boundary of the test field		Foraging bees/minute reduced in both control/treated hive groups but more markedly in treated hives. significant effect of treatment and time ($p<0.001$ for both) on foraging	hives after foraging were not identified. Non-inclusion of raw data. Brood parameters no	

DRAFT
Tier III Effects to Bombus spp.

There were several Tier III studies to characterize the colony-level effects on bumble bees (i.e. various species of Bombus). There was one study available from a registrant submission and two from the open literature (Table 18). As with the higher-tier Apis open literature studies, exposure duration, concentrations tested, and endpoints assessed varied across these studies, and many of the same limitations are noted.

The registrant-submitted study by Wilkins 2014 (49589501) examined effects on bumble bees exposed to flowering rape grown from seeds which were treated with thiamethoxam and seeded at a rate equivalent to 0.02 lb a.i./A. This study included one treated field and two control fields, each with 25 bumble bee colonies (5-week exposure 3-week post exposure monitoring). The author reported no treatment-related colony failures (i.e., a total loss of adult bees or brood), and the mean number of queens produced per colony was similar between the three treatment groups: Control 1 (C1; n=23) contained 18.6 (range 1 to 60); Control 2 (C2; n=21) contained 17.9 (range 1 to 67); and Treatment 1 (Tl; n=22) contained 21.3 (range 1 to 88). The mean numbers of workers and drones produced by all colonies across the treatments were also similar: 54, 47 and 58 workers for C1, C2, and T1, respectively; and, a mean of 33, 34 and 32 drones per colony in C1, C2, and T1, respectively. The author reported that some colonies on site T1 appeared to be increasing in weight until Day 54 and had not started to produce queens, while the other colonies (C1/C2) decreased in weight beginning on Day 47.

For the open literature, Thompson et al. 2015 examined development of bumblebee (B. terrestris audax) colonies where bees had foraged for 5 weeks on flowering winter oilseed rape grown from seed treated with thiamethoxam (as Cruiser™ OSR) using two controls, one treated field. Colony development was evaluated by monitoring the colony mass, forager activity both at the hive and within the crop, and the extent of oilseed rape pollen stored within the colony was analyzed. This study reported an increase[2] in colony mass (13%) relative to controls. No statistically significant effects (see footnote) in foraging activity were observed. Numerically, higher mean numbers of queens/gynes, workers, eggs (2-3x), pupae, and larvae were noted in the thiamethoxam-treated fields. In 2014, Balfour et al. (2017) placed bumblebee colonies (36 per species) adjacent to three large oilseed rape fields (12 colonies per field) planted in 2013 with thiamethoxam treated seeds. Another 36 were in three nearby locations in the same agro-ecosystem, but several kilometers distant from any oilseed rape fields. The study authors report *Bombus* colony growth and reproduction were unaffected by location (distant versus adjacent) following the two month flowering period.

[2] The study author noted the pseudoreplication in the study design and uncertainties in statistical analysis.

Table 18. Thiamethoxam Tier III Registrant Submitted and Open Literature Studies for Bombus sp.

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects[2] (all comparisons made relative to the study's control)	Limitations[2]	Classification Citation (MRID Number)
Thiamethoxam – 0.03 mg a.i./seed *Bombus terrestris* audax	Treated Oilseed Rape Seed (0.02 lb a.i./A)	38-day exposure (Daily assessments for activity within crop)	Field test – 2 controls 1 treated field with 25 colonies/trt group ~2 ha fields drilled 10/6/12 0.02 lb a.i/A	Foraging activity, hive weights, post study hive dissection, pollen analysis	Similar weight gains in colonies. No treatment effects noted for eggs/pupae although mean number in the treated colonies was higher	Two control fields to 1 treated field The treatment hives performed better in number of eggs/pupae than controls LOD not reported for residue analysis. Potential mixing of bees from different treatments/outside sources	Supplemental Wilkins 2014 (49589501)
Thiamethoxam, (Cruiser) 47.6% Clothianidin (Modesto) Imidacloprid (Chinook) *Bombus terrestris*	Treated oilseed rape seed (4.25 kg/ha; 0.029 mg a.i./seed)	38-day exposure 68-day observation	2 control 1 treated field ~ 2 ha and 5 km apart. 20% flower on the treated field colonies placed and moved to monitoring	Colony mass (every 5-8 days), foraging activity (daily during exposure), pollen composition (23-27 days post flowering)	Increase in colony mass (treated); Increased foraging (treated) Higher number of queens/gynes, workers, eggs, larvae	1 treatment replicate Colonies lost to farm/animal damage Uncertain dates of bloom, hive placement	Qualitative Thompson *et al.* 2015:

			75 colonies w/10-20 workers		Lower number of drones	Uncertain if study design adequate to observe effects.	
Cruiser oilseed rape (OSR) seed (% unspecified) *Bombus terrestris audax* *Apis mellifera*	Thiamethoxam-treated oilseed rape seed Blooming plants *Thiamethoxam + clothianidin residues in bee collected pollen and in honey*: *B. terrestris* < 0.1 – 0.49 µg/kg (adjacent sites) <0.1 (distant sites) *A. mellifera* < 0.1 – 1.51 µg/kg (adjacent sites) <0.1 – 0.70 µg/kg (distant sites)	During OSR bloom period *B. terrestris:* 42-58 days (42-44 days for half of the colonies and 56-58 days for the other half starting at exposure initiation) *A. mellifera*: 46-51 days (ca. 1 year starting at exposure initiation)	72 colonies each of honeybees and bumblebees (12 honeybee and 12 bumblebee/site) 3 sites "adjacent" to (< 5 m) thiamethoxam-treated OSR fields and 3 sites "distant" (1.25-4.55 km away) from the nearest OSR field boundary All sites within predominantly agricultural land Honeybee colonies moved to a common site after the exposure period One treatment (adjacent sites) level and one control (distant sites) Pollen sampled during exposure period to determine proportion of OSR sourced pollen Pollen and honey sampled to determine thiamethoxam +	*B. terrestris:* Adult bee populations, # of cocoons, nest weight change, final nest volume *A. mellifera*: Hive weight change, frames of brood, colony survival, queen survival / replacement -- (Yes)	*B. terrestris:* Higher # adult males and workers in adjacent (treatment) colonies *A. mellifera*: Differences in colony weight change between adjacent (treatment) and distant (control) during first three months of the 12 months of the study (treatment weight change more or less than control depending on the month) Negative relationship between mean concentration of thiamethoxam + clothianidin in honey and pollen with cumulative colony weight gain	Single "treatment" level OSR fields also treated with 3 fungicides (picoxystrobin, tebuconazole, and thiophanate-methyl) Potential exposure to other pesticides in surrounding landscape (predominantly agricultural land), including overwintering sites for honeybees (no screen of pollen or honey for other pesticides) No true negative control (thiamethoxam + clothianidin residues were detected in pollen and honey of *Apis* colonies at distant (control) sites; pollen analysis showed OSR foraging by both species at the control sites,	Balfour et al (2017)

			clothianidin concentrations		Fewer frames of brood in 3 adjacent (treatment) colonies in 3 of the 4 final months of the experiment (Dec, Feb, Mar)	potential pesticide use and exposure at control sites may have differed from adjacent (treatment) sites) Single trial	

The three field studies with the mason bee (*Osmia bicornis L.*) were similar in design. All three studies involved exposures of mason bees to thiamethoxam following seed treatments (20.1 µg thiamethoxam/seed) to oil seed rape. Each study took place in a different location in Germany in 2015-2016. The exposure involved parent bees and their offspring. The major limitation of all three studies is that they lacked true replication. Each study included one treated field and one control field, which multiple nesting mason bee sites placed on each. Each nesting site represents a pseudoreplicate. Since there is only one treated field and one control, there is no replication. These studies are considered scientifically valid and useful for characterization purposes. Bees were assessed for hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor. Significant differences in control and thiamethoxam treated sites were observed; however, results differed by location (**Tables 19 and 20**). When all three studies are taken together, it is unclear whether seed treatments of thiamethoxam to oil seed rape impact mason bees.

Table 19. Thiamethoxam Tier III Registrant Submitted and Open Literature Studies for *Osmia bicornis*.

Test Substance – Purity (Test species)	Exposure Matrix (Exposure Level)	Exposure Dur. (Observ. Dur)	Design Elements	Endpoints Assessed -- (Statistical analysis conducted – Yes/No)	Effects (all comparisons made relative to the study's control)	Limitations	Classification Citation (MRID Number)
Thiamethoxam Formulation A9807F	Treated oilseed Rape seeds 20.1 µg thiamethoxam/seed (17.2 ug c.e./seed) Thiamethoxam was measured in several pollen samples collected from the treated site at 3-4 ng a.i./g.	Approximately 1 month	Field test, 1 treated field, 1 control, 8 nesting sites per field.	hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor (Yes)	A significant reduction was observed in female foraging 7 days after exposure (DAE); no other significant differences were detected in this endpoint at different times. None of the other endpoints had significant decreases in the treatment compared to the control. Total nest occupation and cell production were higher in the treatment group compared to the control.	No true replication, only pseudoreplication included in study design	Supplemental 50096602

Thiamethoxam Formulation A9807F	Treated oilseed Rape seeds 20.1 µg thiamethoxam/seed (17.2 ug c.e./seed) Thiamethoxam was detected once in pollen samples collected from the test item treatment field at 1 ng a.i./g and once in nectar samples at 4.1 ng a.i./g.	Approximately 1 month	Field test, 1 treated field, 1 control, 8 nesting sites per field.	hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor (Yes)	The following endpoints were significantly lower in the treatment group compared to the control group: - nest occupation at 6, 9, and 12 days after exposure (DAE), - total cell production, - cell production increases at 6, 9, 12, 15, and 18 DAE, - flight activity at all observations except 18 DAE.	No true replication, only pseudoreplication included in study design	Supplemental 50096604
Thiamethoxam Formulation A9807F	Treated oilseed Rape seeds 20.1 µg thiamethoxam/seed (17.2 ug c.e./seed) Thiamethoxam was detected in one pollen sample at 1 ng a.i./g and clothianidin was detected in one pollen sample at 4 ng a.i./g	Approximately 1 month		hatching rate, nest occupation, cell production, flight and foraging activity, cocoon production, failure and parasitation rate, hatching success and offspring vigor (Yes)	The following endpoints were significantly lower in the treatment group relative to the negative control: - nesting females per unit 15 DAE, females entering the test unit at 3, 9, 13, 15, and 25 DAE, - cocoons per nesting unit and cocoons per hatched female,	No true replication, only pseudoreplication included in study design	Supplemental 50096605

					- male cocoon weight, and - male and female offspring weight.		

Table 20. Significant decrease in endpoint observed in mason bee study (relative to control).

Endpoint	MRID 50096602	MRID 50096604	MRID 50096605
Hatching Rate	No	No	No
Nest Occupation	No*	Yes (3 time points)	Yes (1 time point)
Cell Production	No*	Yes (total)	No
Flight and foraging activity	Yes (increased flight activity at one observation period)	Yes (all but one observation period)	No
Cocoon production	No*	No	No
Cocoon failure and parasitation rate	No	No**	
Hatching success	No	No	No
Offspring vigor	No	No	Yes (decreased weight)

*Nest occupation, cell production and cocoon production were greater in treatment group compared to control.

**Failure rate was higher in control compared to treatment.